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Evaluation of the potential impacts of an influx of CO_2 on subsurface microbial populations using a thermodynamic approach

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Evaluation of the potential impacts of an influx of CO_2 on subsurface microbial populations using a thermodynamic approach

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Summary

The microbial community response to CO_2 injection and the extent to which microbial communities play a role in the geologic sequestration of CO_2 are not well known. In this study we use a thermodynamic approach to scope some potential microbial reactions induced by elevated CO_2 concentrations in subsurface environments, characterised by limited availability and supply rates of energy sources. In these subsurface environments, the energy needed for microbial growth is supplied chemically through thermodynamically favourable electron transfer (redox) reactions. The thermodynamic approach consists of calculating the free energy of these redox reactions which can be used by microbes for their life processes – the energy yield.

As many redox reactions involve protons, in a first approximation the available Gibbs free energies of possible microbially mediated redox reactions are shown as a function of pH and assuming unity activity of reactants and products of each reaction (standard state conditions). Both $H_2 \rightarrow H^+$ and $SO_2 \rightarrow SO_4$ electron donor reactions can have a favourable energy yield for the reduction of $CO_2 \rightarrow CH_4$ at standard conditions. We have also shown that the presence of impurities in the CO_2 waste stream could provide useful energy sources for microbial activity, particularly for SO_2 when coupled to CO_2 reduction.

We have also calculated the energy yields for a range of probable microbially mediated reactions using the fluid composition measured at the Ketzin CO_2 injection site in Germany, where the microbial community response to CO_2 injection has been monitored. Based upon *in situ* concentrations, the influence of thermodynamic constraints on the microbial community composition observed before and after CO_2 injection at the Ketzin saline aquifer has been evaluated.

1 Introduction

The success of carbon capture and storage (CCS) projects depends on the ability of storage sites to contain CO_2, thus mitigating releases to the atmosphere. However, assessments of migration of CO_2 (as a supercritical fluid, gas or dissolved phase) within deep geological systems have tended to only consider inorganic, non-biological processes operating over the relevant timescales (e.g. Gaus et al. 2008; Apps et al., 2010; Keating et al. 2010, Lemieux, 2011). The response of indigenous microbial communities to CO_2 injection and the extent to which microbial communities play a role in the geological storage of CO_2 are largely unknown. For example, CO_2-induced changes in fluid composition may affect the interactions between deep subsurface microorganisms and minerals/fluids; these metabolic reactions may also cause changes in the composition and permeability of the rock formations and/or affecting the well casing (Morozova et al., 2010; West et al., 2011). Thus an understanding of such interactions is a prerequisite important consideration for a full assessment of CO_2 storage.

1.1 SUBSURFACE MICROBIOLOGICAL ECOSYSTEMS

It is well recognised that microbes live in a wide range of subsurface environments, even if growth is strongly constrained by limited nutrient and energy supplies, resulting in very low metabolic rates (West et al. 2011 and references therein). Thus, it is almost certain that microbes can be found in the geological settings considered for CO_2 storage and, consequently, they could be affected by injected CO_2.

Whilst it is extremely unlikely that microbes could survive exposure to supercritical CO_2, because of its physical and chemical properties, many will survive, and may thrive, in contact with wet gaseous or dissolved CO_2 phases (West et al. 2011). Thus, the following impacts can be envisaged:

- CO_2, acting as an oxidant, could be utilised as an energy source by a range of different methanogenic organisms in strongly reducing environments. Methanogenic archea use only hydrogen and CO_2 as energy and carbon sources and do not require any other organic substrates. Thus, it is important to examine the consequences of microbially catalysed methanogenesis. Furthermore, processes of dissolution of iron oxyhydroxides, which have been indicated following CO_2 injection, could lead to the mobilisation of trace metals bound to the iron oxyhydroxides (Kharaka et al. 2006). This may have the potential to impact groundwater quality in situations where potable water supplies overlie sites of deep CO_2 storage.
- An indirect effect of CO_2 on the microbial community results from the alteration of groundwater pH which, in turn, will influence the size and makeup of microbial populations.
- Microbial activity can also affect the permeability of relevant geological formations by the formation of biofilms (Coombs et al, 2010). The formation of biofilms may be impacted by the injection of CO_2, as demonstrated in pilot studies by Wragg et al (2012).
- CO_2 waste streams will contain impurities (such as O_2, H_2S, SO_X and NO_X). These are potential nutrient and energy sources for microbial activity which could alter pH and redox conditions in the subsurface environment (Palandri and Kharaka, 2005).
- Recently, the importance of sulphate-reducing bacteria (SRB) in CO_2 injection sites has been demonstrated at Ketzin, Germany. Here SRB outcompete methanogenic archea after CO_2 injection (Morozova et al., 2010). SRB are known to be involved in accelerating metal corrosion processes, and consequently, are of concern to any CO_2 storage project.

1.2 AIM

In this study, we investigate the influence of thermodynamic constraints on the microbial community composition in potential CO_2 storage scenarios. In subsurface conditions, lithotrophic microorganisms utilise the energy of redox reactions for their life processes, favouring the most energy efficient reactions (the ones with the greatest energy yield). The purpose of this study is to estimate the energy budget for known metabolic reactions relevant to CO_2 storage and determine which of these may provide sufficient energy for microbial usage.

A phased approach was taken which consisted of:

1. Deriving the energy yields of microbially mediated redox reactions, relevant to CO_2 storage scenarios, under standard conditions[1] at T=25°C, 35°C and 70°C, expressed as a function of H^+. From comparisons of the Gibbs free standard energy changes of selected half redox reactions, one may distinguish between thermodynamically feasible and improbable redox reactions at standard conditions and as function of pH.
2. For given *in-situ* conditions relevant to CO_2 storage, to calculate the most energetically favourable redox reaction, which could be utilised by microorganisms as energy source for their life process.
3. The approach was then utilised with information from the Ketzin, Germany CCS demonstration site (Morozova et al., 2010) to compare microbiology results obtained from the thermodynamic approach with actual observations of microbial communities from this site.

1.3 THE THERMODYNAMIC APPROACH

We follow the approach to modelling of microbiological activity in a geological system constrained by the availability of nutrients and energy source, first developed by Grogan and McKinley (1989) for Swiss radioactive waste scenarios and subsequently used in other subsurface environments (e.g. Baker et al., 1998; Jolley et al., 2003; West et al., 2006).

The most important characteristic dictating the progression of a metabolic and any chemical reaction is the amount of energy required or released in the reaction: the energy budget, defined by the Gibbs free energy of reaction (Amend and Shock, 2001). Any reaction that decreases the overall Gibbs free energy of a system is thermodynamically spontaneous.

In order to calculate the available free energy within a system, it is convenient to consider the redox reactions involved in terms of separate half-reactions that explicitly include electrons (e-) written as electron acceptor reactions:

$$A + a\ e^- + b\ H^+ \rightarrow B + c\ H_2O \quad \textbf{(1)}$$

Using thermodynamic principles, it is possible to calculate the energy of the reaction, the Gibbs free energy (ΔG_r), according to:

$$\Delta G_r = \Delta G_r^0 + RT \ln Q \quad \textbf{(2)}$$

where ΔG_r^0 is the standard free energy (kJ mol^{-1}), R is the gas constant (8.31×10^{-3} k J K^{-1} mol^{-1}) and T is the absolute temperature (K).

ΔG_r^0, the standard free energy, can be derived according to:

$$\Delta G_r^0 = \sum_{\mathbf{i}} \vartheta_{\mathbf{i,r}}\ \Delta G_f^0 \text{ (product)} - \sum_{\mathbf{i}} \vartheta_{\mathbf{i,r}}\ \Delta G_f^0 \text{ (reactant)} \quad \textbf{(3)}$$

[1] note: Standard state definition used in Amend and Shock (2001) for solids and water, unit activity of the pure compound at any temperature and pressure; for gases, unit fugacity of the pure gas at any temperature and pressure; for aqueous solutes, unit activity of a hypothetical 1 molal solution referenced to infinite dilution at any temperature and pressure.

where $\vartheta_{i,r}$ is the stoichiometric reaction coefficient of the i^{th} species in reaction r and ΔG_f^0 the Gibbs free energy of formation for each reactant.

The Gibbs free energy (ΔG_r) depends on pressure (P), temperature (T) and composition of the system.

By listing the half-reactions and the Gibbs free energy changes for these reactions normalised by the number of electron (i.e. on an electron equivalent basis) involved in the reaction (unit of kJ mol^{-1} of electrons e-), the ΔG_r /mol e-, it is possible to identify the most favourable electron acceptor and electron donor reactions which, combined, could lead to an exergonic redox reaction (minimising $\Delta G_r = \Delta G_{red} + \Delta G_{ox}$). The half-reaction (written as electron acceptor reactions) with the most negative normalised free energy change will be favoured to proceed as a reduction coupled to the most positive half-reaction running in reverse (as an oxidation).

Previous studies have shown that there is a limit to the minimum energy that can be practically utilised by microbes, generally assumed to be about 15 kJ/mole of electrons transferred (Grogan and McKinley, 1989).

2 Standard free energy and energy yields of microbially mediated redox reactions, relevant to CO_2 storage scenarios

2.1 INTRODUCTION

The Gibbs free energy of a reaction (eq. 2: $\Delta G_r = \Delta G_r^0 + RT \ln Q$) can indicate the direction in which a reaction will proceed. It depends on pressure (P), temperature (T) and composition of the system. The actual energy change will then depend on the concentration of the reactants and products (Q), but in our phased approach, we first consider only the standard free energy ΔG_r^0 of the reaction, without consideration of the other variables (assuming standard[1] state conditions of each reactant and product), except for pH (because pH plays a key role in influencing the most energetically favourable reactions). In the following paragraphs, we then derive the ΔG_r as a function of pH, while keeping reactants and products at unity activity.

2.2 REDOX REACTIONS

In order to examine the feasibility of microbes utilising CO_2 as an energy source, we can consider the fundamental chemical thermodynamic constraints for this process.

In a subsurface environment characterised by low energy fluxes available for microbial life, CO_2 injection represents an important oxidant/electron acceptor in the system. Gas impurities in the CO_2 injection (SOx, H_2S, H_2, CH_4, N_2, NOx, O_2) may also have an influence. A list of some relevant oxidation half-reactions involving these impurities that can be coupled to the reduction of CO_2 is provided in Table 1.

Autotrophic methanogenesis using H_2 as electron donor (half-reaction: A3) and CO_2 as electron acceptor (half-reaction: A1) is a common metabolic process. In many deep environments, sulphide minerals are likely electron donors that have been shown to commonly participate in microbial redox reactions. Under more oxidising conditions the S from minerals such as pyrite (FeS_2) can be oxidised completely to S^{VI} (as SO_4^{2-}) (half-reaction: A2), but, in more reducing environments, a wide range of intermediate S oxidation states may form (half-reaction: A4-5).

2.3 CALCULATION OF GIBBS FREE ENERGIES

The standard[1] Gibbs free energies ΔG_r^0 for a range of probable microbiologically-mediated reactions illustrated in Table 1, have been calculated from the standard free energy of formation (ΔG_f^0), according to equation 3, using the thermodynamic data shown in Table 2 (data available from Amend and Shock, 2001).

The ΔG_r^0 values are then normalised to the number of electrons involved (ΔG_r^0 / mol e-). Table 3 shows the ΔG_r^0 values at T of 25 °C, 37 °C and 70 °C and P of 1 atm.

[1] note: Standard state definition used in Amend and Shock (2001) for solids and water, unit activity of the pure compound at any temperature and pressure; for gases, unit fugacity of the pure gas at any temperature and pressure; for aqueous solutes, unit activity of a hypothetical 1 molal solution referenced to infinite dilution at any temperature and pressure.

Table 1 - Redox-half reactions and equations for calculating ΔG_r, the Gibbs free energy of reaction

Redox half-reactions	Equations for calculating ΔG_r, the Gibbs free energy of reaction
Reduction/electron acceptor reaction	
$CO_2(aq) + 8\ H^+ + 8\ e^- = 2\ H_2O + CH_4(aq)$ **(A1)**	$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[CH_4(aq)][H_2O]^2}{[CO_2(aq)][H^+]^8 a[e-]^8}$
Oxidation/electron donor reactions:	
$FeS_2 + 8\ H_2O = 16\ H^+ + 15\ e^- + Fe^{3+} + 2\ SO_4^{2}$ **(A2)**	$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[Fe^{3+}][SO_4^{2-}]^2[H^+]^{16} a[e-]^{15}}{[FeS_2][H_2O]^8}$
$H_2(aq) = 2\ e^- + 2\ H^+$ **(A3)**	$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[H^+]^2 a[e-]^2}{[H_2(aq)]}$
$SO_2(aq) + 2\ H_2O = 4\ H^+ + 2\ e^- + SO_4^{2-}$ **(A4)**	$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[SO_4^{2-}][H^+]^4 a[e-]^2}{[SO_2(aq)][H_2O]^2}$
$H_2S(aq) + 4\ H_2O = 10\ H^+ + 8\ e^- + SO_4^{2-}$ **(A5)**	$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[SO_4^{2-}][H^+]^{10} a[e-]^8}{[H_2S(aq)][H_2O]^4}$

Table 2 - Tabulation of free energy of formations ΔGf^0 (kJ/mol) for compounds at T 25°C, 37°C and 70°C, data from Amend and Shock 2001

	ΔGf^0		
	T=25 °C	T=37 °C	T=70 °C
$CO_2(aq)$	-386	-387	-392
H^+	0	0	0
H_2O	-237	-238	-241
$CH_4(aq)$	-35	-36	-39
$HFeO_2(aq)$ alias goethite	-423	-424	-426
Pyrite	-160	-161	-163
Fe^{3+}	-17	-14	-4.5
Fe^{2+}	-92	-90	-87
$H_2(aq)$	18	17	15
$SO_4^{2-}(aq)$	-745	-745	-745
$SO_2(aq)$	-301	-303	-309
$S_2O_4^{2-}(aq)$	-600	-601	-604
$S_2O_3^{2-}$	-523	-523	-525
Sulphur(s)	0	-0.39	-1.51
$H_2S(aq)$	-28	-30	-34
HS-	12	11	9
$Acetate^-$	-369	-370	-373

Table 3 - Standard Gibbs free energy ΔG_r^0 and for ΔG_r^0/mol e- (normalised to the number of electron involved) for a range of probable microbiologically mediated reactions at T=25°C, T=37°C and T=70°C

Half reactions	ΔG_r^0 (kJ/mol)			ΔG_r^0 / mol e^- (kJ/mol of transferred electrons)		
	T=25°C	T=37°C	T=70°C	T=25°C	T=37°C	T=70°C
A1	-123	-124	-128	-15	-16	-16
A2	552	562	594	37	37	40
A3	-18	-17	-15	-9	-8	-7
A4	31	35	46	16	17	23
A5	232	237	252	29	30	31

Using the ΔG_r^0 and equation 2, an expression of the free Gibbs energy of reaction ΔG_r as function of pH and under standard condition (i.e. assuming unity activities of reactants and products) has been derived for each of the given electron acceptor and electron donor reactions in Table 1, as shown in Figure 1 and Figure 2. The pH plays a key role in influencing the most energetically favourable reactions.

In both Figure 1 and Figure 2 the ΔG_r/mol e- of the CO_2 half-electron acceptor reaction is plotted on the left y-axis, while all the half-electron donor reactions are plotted against the right y-axis with values in reverse order. There is a minimum energy that can be practically used by microbes, generally assumed to be about 15kJ/mole of electron transferred. This is also illustrated in each figure as a double headed arrow. With the aid of these graphs one may rapidly identify the most favourable half-electron donor reactions to be combined with the half-electron acceptor reaction CO_2 to CH_4 at standard state condition and at a fixed T and pH to give the most exoenergetic redox reaction (minimising $\Delta G_r = \Delta G_{red} + \Delta G_{ox}$). These energetically favourable oxidation half-reactions are represented by the lines above the CO_2 to CH_4 reaction line in Figure 1.

Figure 1 shows that at standard state conditions the electron donor reaction of H_2 oxidation to H^+ when coupled to the reduction of CO_2 to CH_4 in methanogenesis has an energy yield > 15kJ/mole e- at all pH ranges (e.g. under standard conditions, T=25 °C at pH 5, ΔG_r/mol e- = $\Delta G_{CO_2/\ CH_4} + \Delta G_{H_2/H^+} = 13.19 + (-37.4) = -24.21$ kJ/mol e-).

From Figure 1 it is also apparent that under standard conditions and T= 25 °C, at pH 2.5 the oxidation of SO_2 to SO_4 when coupled to CO_2 reduction to CH_4 does not produce sufficient energy yield to drive methanogenesis, with a ΔG_r/mol e-= $\Delta G_{CO_2/\ CH_4} + \Delta G_{SO_2/SO_4} = -1.08 + (-13.01) = -14.09$ kJ/mole e-, smaller than the 15 kJ/mole e- energy yield cut-off for microbial usage. At pH 4 the energy yield of the coupled reactions increases, ΔG_r/mol e- = -22.65 kJ/mole e-, which is above the 15 kJ/mole e- energy yield cut-off for microbial usage. Under the same standard conditions, above pH ~ 4.5 the oxidation of SO_2 to SO_4 produces a greater yield when

coupled to CO_2 reduction to CH_4 than the oxidation of H_2 to H^+ coupled to CO_2 to CH_4 reduction (e.g. at pH 5, ΔG_r/mol e- = $\Delta G_{CO2/\ CH4}$ + $\Delta G_{SO2/SO4}$ = 13.19 + (-41.55) = -28.36 kJ/mol e-, more favourable than ΔG_r/mol e- = $\Delta G_{CO2/\ CH4}$ + $\Delta G_{H2/H^+}$ = 13.19 + (-37.4) = -24.21 kJ/mol e-). It should be noted that these coupled reactions will also result in a decrease in pH, as well as generating CH_4. Consequently, the presence of SO_2 as an impurity in injected CO_2 is potentially an area of uncertainty which requires more detailed evaluation.

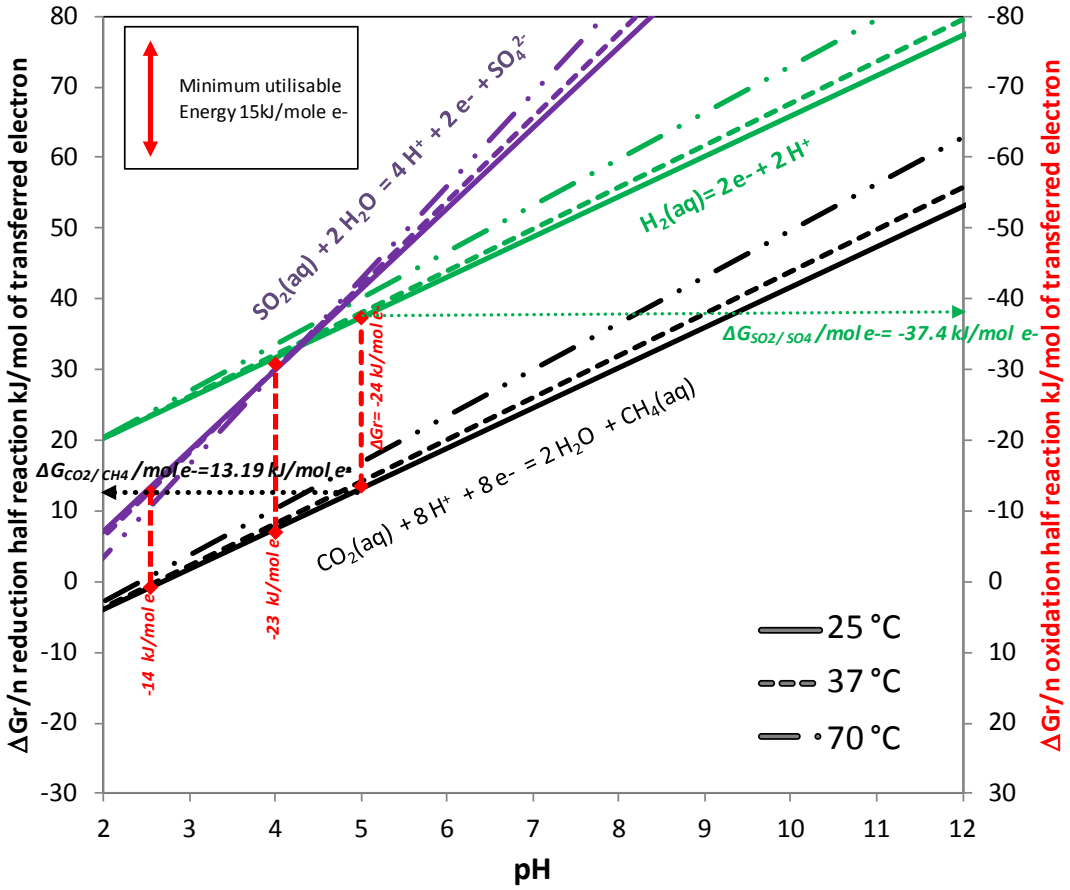

Figure 1 - Variation of the free energy of some potential electron donor half-reactions coupled to electron acceptor half-reaction of CO_2 to CH_4 as a function of pH and varying temperatures, assuming unity activities of reactants and products. CO_2 reduction (black lines) refers to the scale on the left hand side (e.g. $\Delta G_{CO2\text{-}CH4}$/mol e- = 13.19 kJ/mol e^- for pH 5 and T=25 °C), while oxidations (coloured lines) refer to the scale on the right hand scale (e.g. $\Delta G_{H2/H+}$/mol e- = -37.4 kJ/mol e^- for pH 5 and T=25 °C). The electron donor half-reactions represented by the lines above the CO_2 to CH_4 reference line, give a negative ΔG_r of the overall redox reaction, when coupled to the reduction of CO_2 to CH_4.

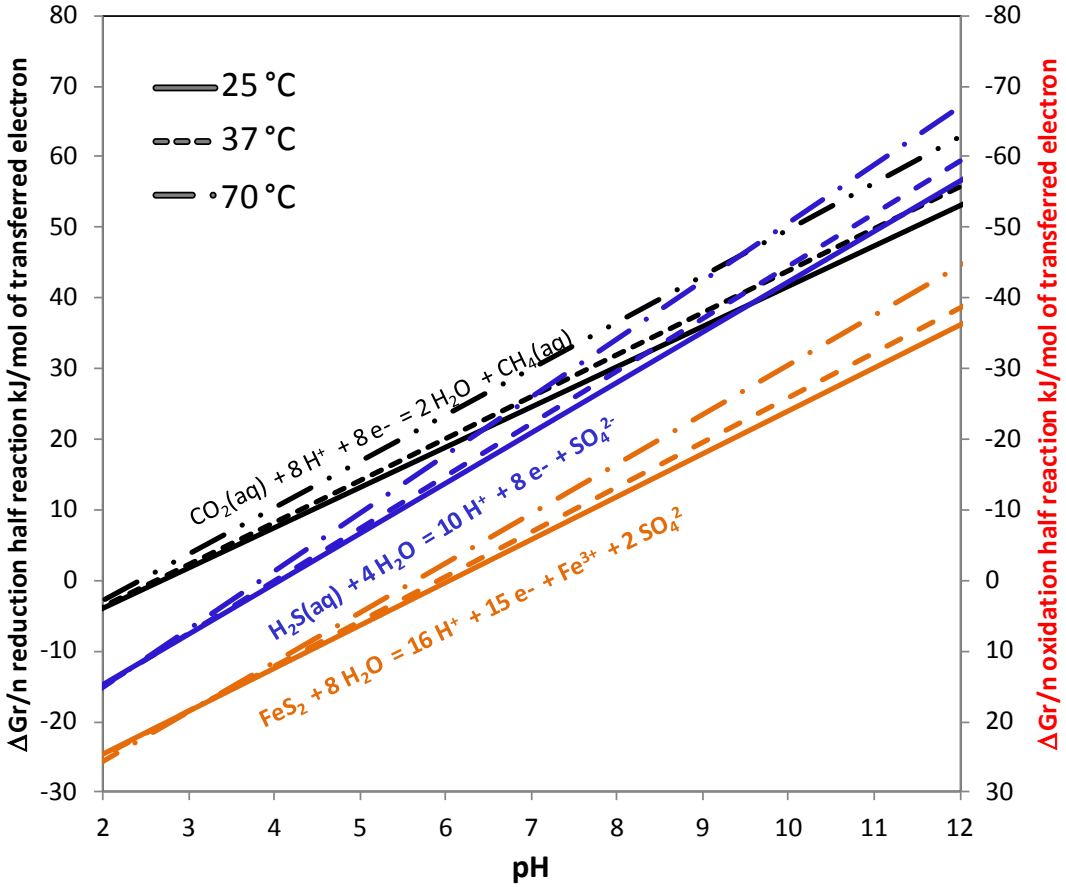

Figure 2 - Variation of the free energy of some potential electron donor half-reactions coupled to CO_2 reduction half-reaction as a function of pH and varying temperatures, assuming unity activities of reactants and products. CO_2 reduction (black lines) refers to the scale on the left hand side, while oxidations (coloured lines) refer to the scale on the right hand scale. The electron donor half-reactions represented by the lines below the CO_2 to CH_4 reference line, give a positive ΔG_r of the overall redox reaction, when coupled to the reduction of CO_2 to CH_4; i.e. they are kinetically inhibited.

Figure 2 shows under standard conditions there is insufficient energy available for microbial methanogenesis from direct pyrite oxidation (e.g. under standard conditions, T=25 °C at pH 5, ΔG_r/mol e- = $\Delta G_{CO_2/\ CH_4}$ + $\Delta G_{FeS_2/Fe^{3+}}$ = 13.19 + 6.3 = +19.49 kJ/mol e-).

Figure 2 also shows the oxidation of the potential gas impurity H_2S, which does not produce sufficient energy for microbial use when coupled to CO_2 reduction at standard state conditions.

3 Application of the bioenergetics thermodynamic approach to the CO_2 storage site in the north eastern Germany near Ketzin to validate the site bioassay

The approach used in Section 3 calculated the Gibbs free standard energy changes of half redox reactions assuming standard state of reactants and products. Consequently, one may distinguish between thermodynamically feasible and improbable redox reactions at standard conditions and as function of pH. These thermodynamic considerations for standard conditions do not prove, however, that a reaction will actually take place. For reactions involving aqueous solutions or gases, the direction in which the reaction will proceed can be determined from the Gibbs free energy of the reaction, once all the activities of the chemical species in the reaction have been evaluated (Amend and Shock, 2001).

Consequently, it is important to calculate and evaluate the significance of these reactions using information from specific and relevant geological settings. As an example, we will use a recent CO_2 injection study at Ketzin, Germany (Morozova et al, 2010). Here, the target reservoir for CO_2 storage is the Triassic Stuttgart formation, at 600-700 m depth. Temperature (T) and pressure (P) of the saline aquifer are approximately 35° C and 6.2 MPa (62 bar), respectively, and salinity 235 mg L^{-1}. Morozova et al. (2010) monitored the effects of the CO_2 exposure on the microbial community from the start of injection for a period of five months, when CO_2 injection stopped. During the study, Morozova et al. (2010) revealed the changes in the deep microbial community of the Ketzin saline aquifer after CO_2 injection, with sulphate reducing bacteria being out-competed by methanogenic archea after the injection of CO_2. The authors stated that the outcome of competition between these trophic groups under acid conditions is regulated more by their pH susceptibility rather than their thermodynamic energy yield. The methanogenic archea have better growth properties than SRB under acid pH.

Our thermodynamic approach aims to validate the bioassay of the study above.

For our calculations, we used the fluid composition pre- and post- CO_2 injection from the observation well 1 (Ktzi 200) as reported by Morozova et al. (2010). These inputs are summarised in Table 4.

Table 4 - Fluid composition in mol L^{-1} from the observation well 1, Ketzin (from Morozova et al., 2010)

Fluid ID	Description	pH	$Acetate^-$ mol L^{-1}	SO_4^{2-} mol L^{-1}	CH_4(aq) mol L^{-1}	CO_2(aq) mol L^{-1}	H_2(aq) mol L^{-1}	N_2(aq) mol L^{-1}	CH_4(aq)/ CO_2(aq)
1	3 months before CO_2 injection	7	0.000615	0.0422	1.1E-05	1.8E-06	6.9E-05	0.00064	5.829
2	2 days after CO_2 arrival	5.3	5.76E-05	0.0376	0.00015	0.0006	0.00129	6.7E-05	0.258
3	2 months after CO_2 arrival	5.3	5.76E-05	0.044	6E-05	0.00028	0.00027	2.4E-05	0.219
4	5 months after CO_2 arrival	5.3	5.76E-05	0.0396	9E-05	0.00062	0.00021	5.3E-05	0.144

3.1 THERMODYNAMIC CALCULATIONS

Using the thermodynamic approach described in Section 2 and data in Table 4, we calculated the *in situ* energy yields for the CO_2/CH_4, H_2/H^+, SO_4^{2-}/HS^-, $CO_2/COOH^-$ redox couples to estimate the most thermodynamically favourable overall redox reaction, given a certain fluid composition and fluid evolution.

Table 5 shows the calculated ΔG^0_r, ΔG_r and ΔG_r/mol e^- of each half-reaction (written as electron acceptor reactions) for the site-specific fluid composition reported in Morozova et al. (2010). By ranking the tabulated free energy changes for each half-electron acceptor reaction normalised by the number of electron involved in the reaction, it is possible to identify the most favourable electron acceptor reaction (the one with the lowest reduction yield) and the most favourable electron donor reactions (the one with the lowest reduction yield), which combined could lead to an exergonic redox reaction (minimising $\Delta G_r = \Delta G_{red} + \Delta G_{ox}$).

Table 5 suggests that, for fluid 1, for example, $SO_4^{2-} + 10\ H^+ + 8\ e^- = H_2S + 4\ H_2O$ is the half-electron acceptor reaction with the lowest normalised free energy change of 18 kJ/mol e- (to proceed as a reduction), that can be coupled to the half-reaction $2\ H^+ + 2\ e^- = H_{2(aq)}$, which has the highest normalised free energy change of 38 kJ/mol e-, but running in reverse (as an electron donor (oxidation) reaction). They would give the most favourable redox reaction $SO_4^{2-} + 2\ H^+ + 4\ H_2(aq) = H_2S + 4\ H_2O$ (B1) (Table 6), which could be utilised potentially by microorganisms as energy sources. A ΔG_r/mol e- = $\Delta G_{red} + \Delta G_{ox}$= 18 + (-38) = -20 kJ/mol e- is above the ΔG/mol e- =15 kJ/mol e^- threshold, which can be practically utilised by microbes.

With fluid 1 composition (before CO_2 injection), thermodynamic calculation predicts that there is not sufficient energy yield (ΔG < 15 kJ/mol e^-) to support methanogenesis by H_2 oxidation (reaction B2: $CO_2(aq) + 4\ H_2 = CH_4(aq) + 2\ H_2O$); the increase in $H_{2(aq)}$ and $CO_2(aq)$ in fluid 2, following CO_2 injection, determines a slightly more favourable ΔG =-16 kJ/mol e^- for methanogenesis via H_2, but SRB might still inhibit methanogenesis.

We also consider the half- reaction of acetate oxidation to CO_2 (Table 6). Acetate oxidation coupled to sulphate reduction, (reaction B3: $SO_4^{2-} + 3\ H^+ + Acetate^- = H_2S + 2\ CO_2(aq) + 2\ H_2O$, Table 6), has an in situ energy yield $\Delta G \leq 15$ kJ/mol e^-.

Acetogenesis, i.e. CO_2 reduction to acetate via H_2 (reaction B4: $CO_2(aq) + 2\ H_2 = 0.5\ Acetate^- + 0.5\ H^+ + H_2O$) does not seem to be feasible with an in situ energy yield ΔG < 15 kJ/mol e^-.

Table 6 shows the calculated energy yields of the possible redox reactions from the combination of these half-reactions. Figure 3 illustrates the changes in Gibbs free energy per mole of transferred electron of each redox reaction in the fluids monitored at the Ketzin saline aquifer before and after CO_2 injection.

These calculations show that reaction B1, Table 6, (sulphate reduction to H_2S via H_2 oxidation), remains thermodynamically favourable over methanogenesis, immediately after CO_2 injection (fluid 2) and up to five months after CO_2 injection (fluid 3 and fluid 4). In reality at Ketzin, Morozova et al. (2010) only showed the predominance of SRB over methanogenic archaea prior to CO_2 injection. After injection, methanogenic archaea predominance was then observed by Morozova et al. (2010).

Thermodynamic calculations do not explain why SRB were outcompeted by methanogenic archea after CO_2 injection. Both microbial groups can tolerate acidic conditions but the archaea do have better growth properties than the SRB (O'Flaherty et al., 1998) which may explain their ability to survive in acidic conditions.

Table 5 - Gibbs free energy ΔG_r^0 , ΔG_r and ΔG_r^0 /n e- normalised to the number of electron involved) for a range of probable microbiologically mediated reactions, calculated using the fluid compositions from the observation well 1, Ketzin (from Morozova et al., 2010)

		ΔG_r^0	ΔG_r	ΔG_r/n e-
CO_2(aq) + 8 H^+ + 8 e- = 2 H_2O + CH_4(aq)	fluid 1, 37°C pH 7	-124	213	27
$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[CH_4(aq)][H_2O]^2}{[CO_2(aq)][H^+]^8 a[e-]^8}$	fluid 2, 37° C pH 5.3	-124	124	16
	fluid 3, 37° C pH 5.3	-124	124	16
	fluid 4, 37° C pH 5.3	-124	126	16
2 H^+ + 2 e- = H_2(aq)	fluid 1, 37°C pH 7	+17	+75	+38
$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[H_2(aq)]}{[H^+]^2 a[e-]^2}$	fluid 2, 37° C pH 5.3	+17	+62	+31
	fluid 3, 37° C pH 5.3	+17	+59	+29
	fluid 4, 37° C pH 5.3	+17	+58	+29
SO_4^{2-} + 10 H^+ + 8 e- = H_2S(aq) + 4 H_2O	fluid 1, 37°C pH 7	-237	145	18
$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[H_2S(aq)][H_2O]^4}{[SO_4^{2-}][H^+]^{10} a[e-]^8}$	fluid 2, 37° C pH 5.3	-237	45	6
	fluid 3, 37° C pH 5.3	-237	44	6
	fluid 4, 37° C pH 5.3	-237	45	6
	Assuming $H_2S(aq)=10^{-7}$ (very low)			
CO_2(aq) +3.5 H^+ + 4e- =0.5 Acetate + H_2O	fluid 1, 37°C pH 7	-36	+134	34
$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[H_2O][Acetate]^{0.5}}{[CO_2(aq)][H^+]^{3.5} a[e-]^4}$	fluid 2, 37° C pH 5.3	-36	+75	19
	fluid 3, 37° C pH 5.3	-	-	-
	fluid 4, 37° C pH 5.3	-	-	-

Table 6 - Possible microbial coupled redox reactions and ΔG_r calculated using the measured concentrations of the fluids at the Ketzin saline aquifer before (fluid 1) and after CO_2 injection (fluids 2 to 4).

B1. $SO_4^{2-} + 2\ H^+ + 4\ H_2(aq) = H_2S(aq) + 4\ H_2O$	($\Delta G_r^{o\,37°C}$ = - 304.96 kJ/mol)	
$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[H_2S(aq)][H_2O]^4}{[SO_4{}^{2-}][H^+]^2[H_2(aq)]^4}$		ΔG_r
	fluid 1 (37°C, pH 7)	-156.45
	fluid 2 (37°C, pH 5.3)	-206.48
	fluid 3 (37°C, pH 5.3)	-190.67
	fluid 4 (37°C, pH 5.3)	-188.05
B2. $CO_2(aq) + 4\ H_2(aq) = CH_4(aq) + 2\ H_2O$	($\Delta G_r^{o\,37°C}$ = - 192.17 kJ /mol)	
$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[CH_4(aq)][H_2O]^2}{[CO_2(aq)][H_2(aq)]^4}$		ΔG_r
	fluid 1 (37°C, pH 7)	-88.85
	fluid 2 (37°C, pH 5.3)	-127.03
	fluid 3 (37°C, pH 5.3)	-111.23
	fluid 4 (37°C, pH 5.3)	-109.97
B3. $SO_4^{2-} + 3\ H^+ + Acetate^- = H_2S(aq) + 2\ CO_2(aq) + 2\ H_2O$	($\Delta G_r^{o\,37°C}$ = - 165 kJ /mol)	
$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[CO_2(aq)]^2[H_2S(aq)][H_2O]^2}{[SO_4{}^{2-}][H^+]^3[COOH^-]}$		ΔG_r
	fluid 1 (37°C, pH 7)	-122.80
	fluid 2 (37°C, pH 5.3)	-116.81
	fluid 3 (37°C, pH 5.3)	-121.19
	fluid 4 (37°C, pH 5.3)	-116.71
B4. $CO_2(aq) + 2\ H_2(aq) = 0.5\ Acetate^- + 0.5\ H^+ + H_2O$	($\Delta G_r^{o\,37°C}$ = - 70 kJ /mol)	
$\Delta G_r = \Delta G_r^{oT} + RTln\frac{[Acetate^-]^{0.5}[H^+]^{0.5}[H_2O]}{[CO_2(aq)][H_2(aq)]^2}$		ΔG_r
	fluid 1 (37°C, pH 7)	-16.84
	fluid 2 (37°C, pH 5.3)	-44.85
	fluid 3 (37°C, pH 5.3)	-34.76
	fluid 4 (37°C, pH 5.3)	-35.69

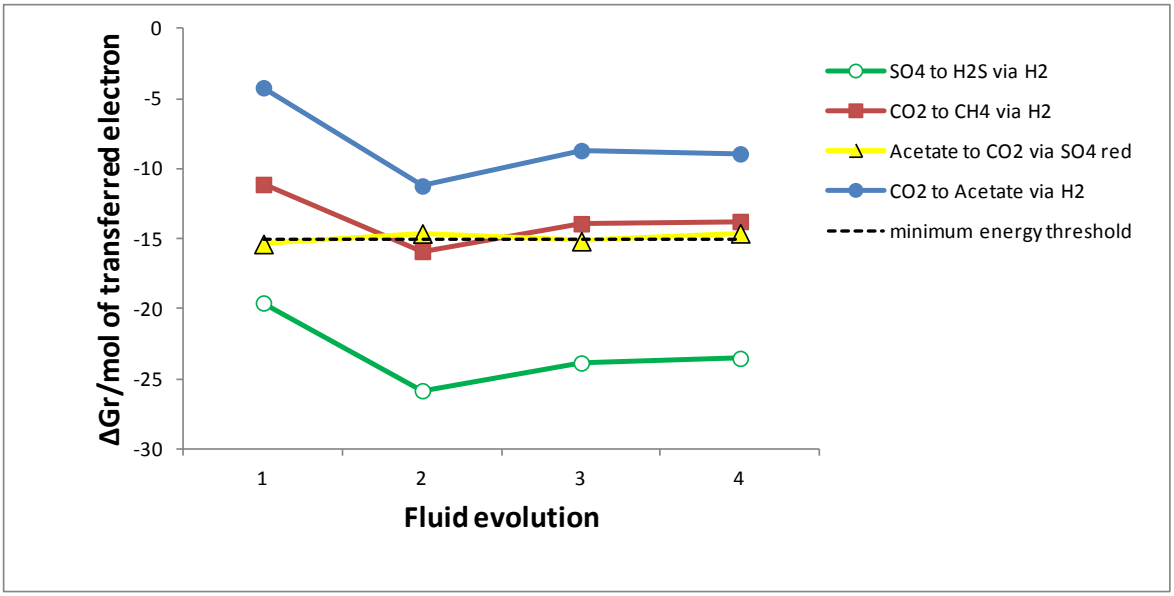

Figure 3 - The Gibbs free energy/ mol of transferred electrons for a range of possible microbial processes in the fluids monitored at the Ketzin saline aquifer before (fluid 1) and after CO_2 injection (fluids 2 to 4) calculated from the measured concentrations of reactants and products. The dashed line indicates the minimum useable energy per mol of electrons transferred.

4 Conclusions

This report has shown that a thermodynamic approach to evaluating energy yield for microbial usage from redox couples relevant to the geological storage of CO_2 is possible and can provide useful indications of the significance of microbial activity on storage.

We have derived the energy yields of microbially mediated redox reactions, relevant to the CO_2 storage scenarios, under standard conditions (unity activity of reagents and products) at T=25°C, 35°C and 70°C, expressed as function of H^+. From comparisons of the Gibbs free standard energy changes of selected half redox reactions, one may distinguish between thermodynamically feasible and improbable redox reactions at standard conditions and as function of pH. Both $H_2 \rightarrow H^+$ and $SO_2 \rightarrow SO_4$ electron donor reactions can have a favourable energy yield for the reduction of $CO_2 \rightarrow CH_4$ at standard conditions. We have also shown that the presence of impurities in the CO_2 waste stream could provide useful energy sources for microbial activity, particularly for SO_2 when coupled to CO_2 reduction.

We have also calculated the energy yield for a range of probable microbially mediated reactions using the fluid composition measured at the Ketzin CO_2 injection site in Germany. Thermodynamic calculations indicate that in the competition between sulphate reducing bacteria (SRB) and methanogenic bacteria for hydrogen, for the saline aquifer at Ketsin the most energetically favourable reaction is the sulphate reduction to H_2S via H_2, either before and after CO_2 injection. Methanogenesis increases the free energy yield following CO_2 injection, but sulphate reduction via H_2 remains more favourable. When comparing the thermodynamic predictions with the range of organisms detected at Ketzin, there is agreement for the fluid before CO_2 injection, i.e. SRB prevail over the methanogenic Archaea. However, the archaea outcompete the SRB immediately after CO_2 injection and this does not seem related to the amount of energy available (as calculated by this study). Other factors are coming into play and it appears (from past literature) that the inherent strength of the archaea in extreme conditions is proving to be a significant advantage when CO_2 is injected into this environment (at least for the first few months after injection).

Taken together, the work described in this report suggests that:

- The thermodynamic approach is useful for broadly evaluating potential CO_2 storage environments for their ability to support microbial populations;
- The approach indicates potential groups of organisms that may be found in a particular environment and also those groups that may be supported by the injection of different CO_2 waste streams;
- Thermodynamic evaluation is dependent on range and quality of mineralogical/geochemical/hydrochemical information from a site;
- Thermodynamic evaluation is not a substitute for actual observations of microbial populations and their impacts. It is a complementary tool that is an effective ‘first’ evaluation of the microbiology of a particular CO_2 storage project.

The next phase of modelling will expand the number of simulated microbial reactions, also considering the role of iron oxyhydroxides and sulphides to a greater extent and will test different CO_2 storage settings where geochemical data are available.

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